under metabolic control via redox regulation through changes in NAD^+/NADH (Schwer and Verdin, 2008). However, histone acetylation is but one part of a larger story. Recent work identifies two metabolic enzymes regulated by acetylation, phosphoenolpyruvate carboxykinase (Lin et al., 2009) and carbamoyl phosphate synthetase (Nakagawa et al., 2009). In the end, it is not surprising that many mechanisms controlling gene activity involve metabolites. The challenge will be to identify which molecules in our biochemistry textbooks have signaling functions and which ones modify metabolic networks in a way that alters the balance of this homeostatic system, and to decipher how cancer cells tweak metabolism and gene expression in their favor. Identifying receptors for such compounds or accurately monitoring steady-state concentrations for all cellular metabolites, plus their fluxes, will be difficult. Yet the field will eventually need to develop a systems level understanding of metabolism. As intractable and frustrating as small molecules can be to experimentalists, the future clearly holds plenty of challenges, but also promises great rewards.

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Breaking Up Just Got Easier to Do

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The SLX4 protein functions as a platform for catalytic subunits of structure-specific endonucleases. Findings reported in *Cell* (Fekairi et al., 2009; Svendsen et al., 2009) and in *Molecular Cell* (Andersen et al., 2009; Muñoz et al., 2009) now identify the human SLX4 and show that in association with the SLX1 endonuclease it directs the symmetric cleavage and resolution of Holliday junctions.

The homologous recombination model proposed by Robin Holliday in 1964 envisioned a crossed-strand structure, the Holliday junction (HJ), as the final intermediate in exchange between two homologous DNA duplexes. This model, combined with genetic data on meiotic crossover formation, predicted an endonuclease that would cleave an HJ symmetrically on opposing single DNA strands, generating products that could be ligated (Figure 1). Moreover, the ligation reaction should not add or delete nucleotides, so the resulting recombinant products would preserve the DNA sequences at the crossover junction. Once it was possible to make HJ substrates in vitro, the RuvC endonuclease from the bacterium *Escherichia coli* became the first resolvase to be identified (Dunderdale et al., 1991), and it seemed it would only be a short time before the eukaryotic HJ resolvases were known.

However, biology has a way of complicating matters, and the road to the eukaryotic resolvase has been marked by dead end exits and incorrect turn offs. In fact, it has turned out that the HJ substrate arises from events other than meiotic recombination between homologous chromosomes, and so the cell has found it necessary to devise both multiple resolvases and multiple ways to eliminate an HJ. A further level of complexity arises from the diversity of the organisms under study, ranging from yeast to humans. A given species may preferentially use one of the several resolvase complexes in meiosis, such that a mutant in one organism may have a strong meiotic crossover defect, whereas the same mutant in a different organism is barely discerned as defective. As if this were not enough, cells have also devised a mode of resolution, called dissolution, that involves a helicase-toposiomerase complex and not structure-specific endonucleases. These facts serve to highlight the importance of the HJ structure and the need to resolve it accurately. In that sense, the discovery of another resolvase is not entirely surprising. What is surprising from the new work is that the key player, SLX1, had already been identified as a structure-specific endonuclease in *S. cerevisiae*,

but with a preference for 5′ flap structures. Although yeast SLX4 had been known to cleave HJs, it did not cut them symmetrically.

This story begins with the discovery of the *SLX* genes (Mullen et al., 2001) as mutants that require the RecQ helicase Sgs1 for survival. Given that one mode of unhooking DNA strands linked by a HJ is dissolution through the combined action of the RecQ helicase Sgs1 or the human ortholog BLM together with its topoisomerase partner Top3 or TopoIIIα, it now seems logical that the SLX proteins would act on branched DNA structures. Indeed, one pair of SLX proteins is Mus81-Mms4 (MUS81-EME1 in *S. pombe* and humans), and this complex has been shown to cleave branched DNA structures such as flaps and HJ molecules. Another complex is Slx1-Slx4, but in vitro the *S. cerevisiae* proteins did not cleave an HJ in a symmetric fashion, the mutants did not have a strong meiotic phenotype, and the Slx4 protein did not seem to be conserved beyond yeast. Paradoxically, the Slx1 protein, which has the catalytic endonuclease activity, is conserved. Equally important was the observation that the phenotypes of *S. cerevisiae slx1* and *slx4* mutants are not identical when it comes to DNA damage sensitivity, suggesting that Slx4 could function independently of Slx1 in DNA damage repair and genome maintenance (Fricke and Brill, 2003). Now, in the current issues of *Cell* and *Molecular Cell*, four groups (Fekairi et al., 2009; Svendsen et al., 2009; Muñoz et al., 2009; Andersen et al., 2009) have identified the human ortholog of SLX4 and show that with SLX1 the complex has HJ resolvase activity. The groups also propose that SLX4 acts as a platform for several endonucleases for HJ resolution, DNA repair, and interstrand crosslink repair through its different protein interaction domains. This architecture allows SLX4 to control substrate nicking and recruitment of two different endonucleases simultaneously to one damaged substrate.

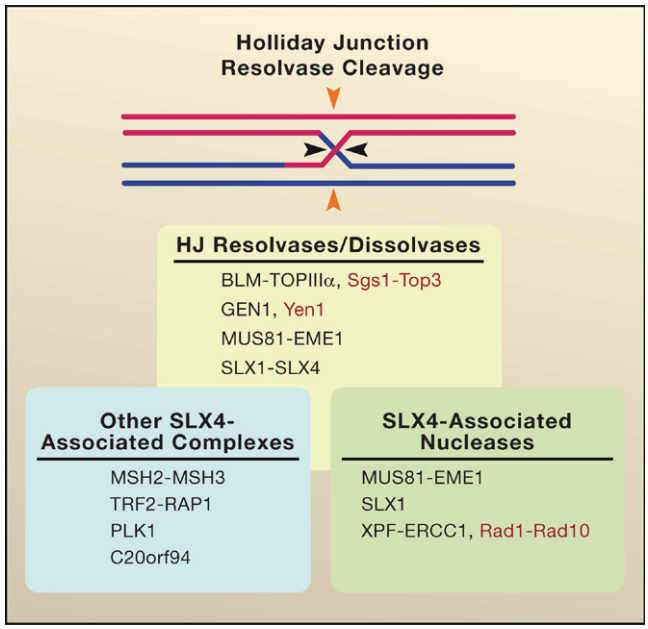

Figure 1. Resolving Holliday Junctions

(Top) A Holliday junction (HJ) DNA structure is shown. Cleavage in the vertical direction (orange arrowheads) gives crossover chromosomes, whereas cleavage in the horizontal direction (black arrowheads) results in noncrossover products. (Bottom) Summary of the HJ resolvases and dissolvases (yellow box), endonucleases (green box), and other factors (blue box) found to bind to SLX4. If named different than the human protein, the protein name in the budding yeast *Saccharomyces cerevisiae* is designated in red.

The human SLX4 protein is identified through in silico analyses by Fekairi et al. (2009), starting off with the alignment of the fungal Slx4 proteins to identify a conserved C-terminal domain that has homology to the *Drosophila* MUS312 protein and the vertebrate protein BTBD12. MUS312 binds to MEI9, the *Drosophila* form of the endonuclease XPF (Rad1 in *S. cerevisiae*). A second analysis with MUS312 again identifies BTBD12 and confirms homology to the MSU312-MEI9 interaction domain. A second group approached SLX4 by looking at protein interactions with BTBD12, drawn to it because it is a target of the ATM/ATR kinases (Svendsen et al., 2009). As an aside, it should be noted that Mec1 and Tel1, the yeast ATR and ATM orthologs, phosphorylate the *S. cerevisiae* Slx4 protein (Flott et al., 2007). In related studies, Muñoz et al. (2009) and Andersen et al. (2009) identify human BTBD12 through similar in silico analyses. Given that the *S. cerevisiae* Slx1-Slx4 complex is reported to cleave HJ structures (Fricke and Brill, 2003) and the *Drosophila* MUS312-MEI9 complex is required for meiotic crossovers, the Gaillard, Harper, and Rouse groups examine the activity of human SLX1-SLX4 complex for the ability to resolve HJs. In some cases, the HJ substrate cleavage is not completely symmetric, particularly when full-length SLX4 is used from human cells, because additional endonucleases that interact with SLX4, such as MUS81-EME1, compromise the cleavage reaction. But when SLX1-4 purified from *E. coli*, an SLX4 fragment that does not interact with MUS81-EME1, or immunoprecipitates of tagged SLX1 are used, symmetrical cleavage of migrating and static HJ substrates and subsequent ligation is observed (Fekairi et al., 2009; Svendsen et al., 2009). This is similar to that observed with the human GEN1 resolvase (Ip et al., 2008). These findings suggest a modular organization of SLX4 that allows association with different endonuclease partners to regulate cleavage of damaged or branched substrates. It explains the independent phenotypes of the *slx1* and *slx4* mutants in yeast and the use of multiple nucleases in interstrand crosslink repair in a coordinated fashion, and could explain why some resolvase enzymes are more predominant in some species compared to others. It also offers new and expanding possibilities for SLX4 as a coordinating platform for branched DNA structure recognition; additional partners of SLX4 include the HDM protein of *Drosophila* required for a subset of meiotic crossovers (Joyce et al., 2009), the mismatch repair proteins MSH2/MSH3 (Svendsen et al., 2009), and the telomere proteins TRF2 and RAP1 (Svendsen et al., 2009) (Figure 1).

It will be important to identify the SLX1 protein in *Drosophila* and show that a mutant in the gene has a meiotic crossover phenotype similar to that of the *mus312* mutant. It is not known how or indeed if the MEI9 protein cleaves HJs, or whether the involvement of MEI9 in meiotic recombination is due

to its association with SLX4/MUS312 as a factor that works in concert with a SLX1 endonuclease. Additional issues for future studies are the precise role of SLX4 phosphorylation by the DNA damage checkpoint kinases and how this might affect its association with the different endonucleases, how SLX4 recognizes and binds different DNA structures, and the stoichiometry of SLX4 and its associated nucleases. The latter issue is particularly important at HJs where two symmetric cuts are needed for proper resolution. For example, how many endonucleases can a single SLX4 molecule bind simultaneously through its different motifs to affect regulated cutting of damaged DNA substrates? And how does SLX4 toggle between promoting two cuts on one DNA strand, such as might be needed for interstrand crosslink repair, and one cut each on paired DNA strands for HJ resolution?

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A Positive Spin on the Centromere

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The properties of centromeric nucleosomes have been the subject of considerable debate and controversy. Furuyama and Henikoff (2009) now provide surprising evidence that centromeric nucleosomes wrap DNA in an orientation that is opposite to that of canonical nucleosomes.

The centromere is a region of the chromosome that directs assembly of the kinetochore, a large proteinaceous complex that mediates chromosome attachment to microtubules. The equal partitioning of chromosomes in mitosis and meiosis relies on the faithful propagation of centromere location during DNA replication. Although centromeres are associated with specific DNA sequences, these sequences are not evolutionarily conserved. In budding yeast, centromeric DNA is characterized by an ~125 base pair DNA motif, whereas centromeric DNA in fission yeast, plants, and mammals is composed of megabases of repetitive α satellite DNA. Thus, something other than DNA sequence must confer centromeric behavior. A favored candidate has been the replacement of the canonical histone H3 with the histone variant CenH3. The key unanswered question is this: how does CenH3 substitution modify chromatin structure? According to findings presented by Furuyama and Henikoff (2009), the presence of CenH3 may make DNA loop around centromeric nucleosomes with a right-handed orientation—the opposite of that observed for canonical nucleosomes.

The composition and structure of nucleosomes with CenH3 has been the subject of much debate. In humans, the majority of centromeric nucleosomes contain an equal number of copies of CenH3, H4, H2A, and H2B, with minor fractions containing both CenH3 and H3 (Foltz et al., 2006). In vitro experiments also show that CenH3 can replace H3 to form a complex composed of equimolar amounts of CenH3, H4, H2A, and H2B with two copies of each histone being required for the apparent molecular weight of 200 kDa (Yoda et al., 2000). This contrasts with data demonstrating that centromeric chromatin in the fruit fly *Drosophila melanogaster* forms structures, referred to as hemisomes, that are composed of single copies of CenH3, H4, H2A, and H2B (Dalal et al., 2007). And yet another variation exists in the budding yeast. Their centromeric nucleosomes may be hexameric, consisting of two copies of CenH3 (Cse4), H4, and Scm3, a nonhistone protein that targets Cse4 to centromeres (Camahort et al., 2007; Mizuguchi et al., 2007). Scm3 also assembles CenH3 into centromeres in fission yeast, but is not likely to be incorporated into centromeric